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Food Safety and Inspection Service, Office of Public Health Science

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Title: Screening and Confirmation of β -Lactam Antibiotics by HPLC-MS/MS		
Revision: 02	Replaces: CLG-BLAC.01	Effective: 02/08/2007

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A. INTRODUCTION

1. Theory

Beta-Lactams are extracted from tissues using acetonitrile/water. Interfering substances are removed using solid phase extraction (SPE). The eluate is reduced in volume and examined for the presence of β -Lactams by LC/MS/MS using a triple quadrupole mass spectrometer under electrospray ionization (ESI) conditions. Analytes are identified and/or confirmed by comparison against external or matrix-matched standards.

2. Applicability

This method is applicable for confirming the following β -Lactams in bovine kidney and muscle: Ampicillin ≥ 10 ppb; Nafcillin ≥ 20 ppb; Cefazolin, Desfuroylceftiofur cysteine disulfide metabolite of Ceftiofur (DCCD), Penicillin G each ≥ 50 ppb; and Desacetyl Cephapirin ≥ 100 ppb.

This method may also be used to screen for Amoxicillin and Cloxacillin at ≥ 10 ppb and Dicloxacillin and Oxacillin at ≥ 50 ppb.

B. EQUIPMENT

1. Apparatus

Note: Equivalent apparatus may be substituted.

- a. Centrifuge tube - 50 mL, polypropylene, Falcon, Cat. No. 35-2070, Becton Dickinson Labware.
- b. Vortex mixer - Vortex Genie 2, Scientific Industries.
- c. Shaker - Eberbach.
- d. Centrifuge (capable of 3400 G and refrigeration to 4 °C) - Model GP8R, IEC.
- e. SPE cartridge - BakerBond® C₁₈, Cat. No. 7020-27.
- f. Vortex Evaporator - TurboVap LV, Zymark.
- g. 15 mL glass culture tube (for Zymark evaporator) - Kimble P/N 73790-15.
- h. Syringe - 1.0 mL polypropylene.
- i. Pasteur pipets - disposable glass, 9 inch.
- j. Filter, Syringe - 0.2 μ m, Gelman Acrodisc (PVDF, P/N 4450T or PTFE, P/N 4423T).
- k. Autosampler vials - 2 mL, with graduated marking, Cat. No. 957009, PJ Cobert.
- l. Centrifuge - capable of 16000 rpm (approx. 23000 g) for 1.5 mL centrifuge tubes, Eppendorf Model 5417C, Brinkmann Instruments.

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- m. Microcon ultracentrifuge filters - 3 Kda cutoff, Millipore, Cat. No. 42403.
- n. Autosampler limited volume vials - polypropylene (500 μ L or 750 μ L capacity), P/N 951511, P.J. Cobert.
- o. Freezers - capable of maintaining temperatures < -10 °C for sample and standards storage.
- p. Blender - Waring Products Division.
- q. Robot Coupe® processor - Robot Coupe U.S.A., Inc.

2. Instrumentation

Note: Equivalent instrument may be substituted.

- a. Triple quadrupole mass spectrometer - Thermo-Finnigan TSQ Quantum.
- b. High Performance Liquid Chromatograph (HPLC) - Thermo-Finnigan Surveyor.
- c. HPLC Column - 4.6 x 50mm (3 μ m particle size) YMC ODS-AQ, Waters, Cat. No. AQ12S030546WT with 4.0 x 20mm (3 μ m particle size) YMC ODS-AQ guard column, Waters Cat. No. AQ12S030204WDA.

C. REAGENTS AND SOLUTIONS

Equivalent reagents and solutions may be substituted for the following unless otherwise indicated:

1. Reagents

- a. Acetonitrile (ACN) - HPLC grade, Cat. No. 015, Burdick & Jackson.
- b. Methanol (MeOH) - HPLC grade, Cat. No. 230-4, Burdick & Jackson.
- c. Formic acid - ACS reagent grade, Cat. No. F-4635, Sigma-Aldrich.
- d. Deionized water.
- e. Hexane - Cat. No. H303-4, Fisher Scientific.

2. Solutions

- a. 50:50 MeOH/Water:
Combine equal volumes of MeOH and deionized water, and mix.
- b. HPLC Aqueous Mobile Phase (0.1% formic acid):
Add 1.0 mL of formic acid to a 1.0 L volumetric flask. Dilute to volume with deionized water. Solution is stable for 6 months at room temperature.
- c. HPLC Organic Mobile Phase (50% MeOH, 50% ACN, 0.1% formic acid):
Add 500 mL of MeOH and 500 mL of ACN to a 1.0 L volumetric flask and mix.
Add 1.0 mL of formic acid to the flask and mix. Solution is stable for 6 months at

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room temperature.

D. STANDARDS

Note: Equivalent standard/solutions may be substituted for the following.

1. Reference Standards

The following compounds are available commercially from sources cited:

- a. Amoxicillin, Ampicillin, Cefazolin, Cloxacillin, Nafcillin, Oxacillin, Penicillin G (benzylpenicillin), and Penicillin V (phenoxymethylpenicillin): U.S. Pharmacopeia, Rockville, MD.
- b. Dicloxacillin: Sigma-Aldrich, Milwaukee, WI.

Note: The following metabolites were produced in limited quantities by sources cited, but are not available commercially.

- c. Desfuroylceftiofur cysteine disulfide (DCCD): Pharmacia, Kalamazoo, MI.
- d. Desacetyl Cephapirin: Intervet, Vienna, Austria.

2. Standard Solutions

- a. Stock Standards (100 $\mu\text{g/mL}$):

Prepare individual β -Lactam stock solutions. Weigh 1.0 mg (or an equivalent amount when corrected for purity) into a 10 mL volumetric flask. Dissolve and dilute to volume with 50:50 (v/v) MeOH/water. For some analytes (e.g., cefazolin and amoxicillin) the solution may need to be sonicated for 30 to 60 minutes to completely dissolve solids.

- b. Penicillin V Internal Standard (20 $\mu\text{g/mL}$):

Pipet 2.0 mL Penicillin V stock standard into a 10 mL volumetric flask and dilute to volume with 50:50 (v/v) MeOH/water.

- c. Intermediate Mixed Standard:

Pipet 1.0 mL of each of the Amoxicillin, Ampicillin, and Cloxacillin stock standards into a 10 mL volumetric flask. Add 2.0 mL of Nafcillin stock solution and dilute to volume with 50/50 (v/v) MeOH/water.

- d. Fortification Standard:

This solution is used to prepare the external standard and to fortify the positive control and matrix-matched standard.

Pipet 2.0 mL of Intermediate Mixed Standard into a 50 mL volumetric flask. Add 1.0 mL each of Desfuroylceftiofur cysteine disulfide, Cefazolin, Penicillin G, oxacillin, and Dicloxacillin stock standards and 2.0 mL of Desacetyl Cephapirin stock standard to the flask. Dilute to volume with 50:50 (v/v) MeOH/water.

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Analyte concentrations of individual analytes in this standard and their equivalent concentrations in the positive control and samples are listed below.

β - Lactam Analyte	Concentration in Fortification Standard	Concentration in Control*
Amoxicillin, Ampicillin, Cloxacillin	0.4 $\mu\text{g/mL}$	10 ppb
Nafcillin	0.8 $\mu\text{g/mL}$	20 ppb
Cefazolin, DCCD, Dicloxacillin, Penicillin G, oxacillin	2 $\mu\text{g/mL}$	50 ppb
Desacetyl Cephapirin	4 $\mu\text{g/mL}$	100 ppb

* Based on addition of 50 μL fortification standard to 2 g blank tissue.

- e. External Standard:
Add 100 μL of Penicillin V Internal Standard (D.2.b) and 50 μL of Fortification Standard (D.2.d) to an autosampler vial. Dilute to the 1 mL mark on vial with deionized water. Prepare fresh daily.
- f. Storage and Stability: Solutions are stable for 2 months when stored in glass bottles at $< -10\text{ }^{\circ}\text{C}$.

E. SAMPLE PREPARATION

- 1. Sample Preparation
 - a. Place kidney or muscle tissue into $< -10\text{ }^{\circ}\text{C}$ freezer immediately upon receipt.
 - b. Partially thaw frozen tissue to allow removal of fatty and/or connective tissue with knife.
 - c. Thoroughly blend muscle samples in a food processor after removal of excess fat. Homogenize kidneys to a semi-liquid consistency using a blender or food processor. Transfer blended tissues to a small plastic bag, seal, and immediately replace into $< -10\text{ }^{\circ}\text{C}$ freezer. Test sample analysis should be started as soon as possible after preparation to minimize possible losses of analyte due to enzymatic degradation.
- 2. Test Sample Preparation and Cleanup
 - a. Weigh $2.0 \pm 0.1\text{ g}$ of frozen tissue into a 50 mL polypropylene centrifuge tube. Return unused portion to $< -10\text{ }^{\circ}\text{C}$ freezer.

Note: At this time, weigh three 2 g portions of blank tissue (tissue without any of detectable levels of β -lactams) into 50 mL polypropylene centrifuge tubes. One

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is used as a negative control, the others to prepare a positive control (recovery) and a matrix matched standard.

Note: A matrix matched standard is the extract of a blank tissue of the same matrix type as the test sample, fortified just prior to LC/MS analysis.

- b. Allow tissue to fully thaw.

Prepare the positive control at this time by fortifying one matrix blank with 50 μ L of Fortification Standard. Vortex for 10 - 15 sec. and allow to stand for approx. 5 minutes before addition of extraction solvents.
- c. Add 2.0 mL of deionized water and 8.0 mL of acetonitrile to the tube, and cap.
- d. Vortex the tube briefly then place on shaker for 5 minutes at high speed.
- e. Centrifuge tube at about 3400 G, at approx. 4 °C, for 15 minutes.
- f. Decant the aqueous acetonitrile extract into a second 50 mL Falcon tube.
- g. For muscle extracts, the following additional cleanup steps are required:
 - i. Add 5 mL of hexane to the tube and either vortex for 1 minute or place on shaker for 5 minutes at high speed.
 - ii. Centrifuge tube at about 3400 G, at approx. 4 °C, for 15 minutes.
 - iii. Siphon off the upper hexane layer using a Pasteur pipette.
- h. Prepare SPE cartridge:
 - i. Add 5 mL of acetonitrile to the cartridge and allow to flow through by gravity. (Gentle vacuum may be needed to initiate flow).
 - ii. Add 5 mL of deionized water to the cartridge and allow to flow through by gravity. Repeat with second 5 mL rinse, stopping flow when water level is just above the top of the SPE bed.
 - iii. Place a 15 mL glass culture tube into the SPE sample manifold to collect eluate.
- i. Apply sample extract to the SPE cartridge. Allow to drain through under gravity flow. When all the extract has eluted under gravity, apply gentle vacuum to elute the residual interstitial solution.

Stopping point: Sample can be left overnight in refrigerator prior to evaporation.
- j. Remove eluate collection tube from the manifold and place in vortex evaporator, with a water bath temperature at 38 - 41 °C. Evaporate to a volume < 1 mL under maximum nitrogen flow. (This may take 50 - 70 minutes).
- k. Transfer eluate using a Pasteur pipette to a syringe fitted with a 0.2 μ m syringe filter. Filter the eluate directly into an LC autosampler vial to which 100 μ L of Penicillin V Internal Standard has been added.

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Fortify the Matrix-matched standard at this time. Add 50 μ L of Fortification Standard to the autosampler vial containing the blank eluate reserved for this purpose.

- l. Dilute solution in each vial to the 1 mL mark with deionized water.
- m. Load sample onto ultracentrifuge filter. Centrifuge at about 16000 rpm (about 23000 g) for 20 - 90 min (or per manufacturer specifications).
- n. Transfer filtrate to limited volume vial (500 μ L or 750 μ L) for analysis.

Stopping point: Samples are stable for up to three days when stored in refrigerator.

F. ANALYTICAL PROCEDURE

1. HPLC Operating Parameters

Note: Recommended values. Settings may be adjusted, if necessary, to optimize HPLC separation.

- a. Injection volume: 100 μ L.
- b. Elution gradient:

Time (min)	Flow rate (mL/min)	Aqueous Mobile Phase (%)	Organic Mobile Phase (%)
0.00	0.30	100	0
22.00	0.30	0	100
22.10	0.50	0	100
27.00	0.50	0	100
28.00	0.50	100	0
31.00	0.50	100	0
31.10	0.30	100	0
40.00	0.30	100	0

- c. Divert valve timing:
 - i. 0.0 - 4.5 minutes: waste
 - ii. 4.5 - 24.0 minutes: Mass spectrometer
 - iii. 24.0 - 40.0 minutes: waste

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2. Mass Spectrometer Setup

- a. Program the mass spectrometer to collect the product ions:

Analyte	Parent Mass	Product Ion	Scan Width
Desacetyl Cephapirin	382	292	2.0
		226	2.0
		112	2.0
Amoxicillin	366	349	2.0
		208	2.0
		114	2.0
Desfuroylceftiofur cysteine disulfide (DCCD)	549	366	2.0
		241	2.0
		183	2.0
Ampicillin	350	192	2.0
		160	2.0
		106	2.0
Cefazolin	455	323	2.0
		156	2.0
Penicillin G	335	176	2.0
		160	2.0
Oxacillin	402	287	2.0
		243	2.0
		186	2.0
Penicillin V (internal standard)	351	192	2.0
		160	2.0
Cloxacillin	436	321	2.0
		220	2.0
		178	2.0
Nafcillin	415	256	2.0
		199	2.0
Dicloxacillin	470	254	2.0
		212	2.0
	472	256	2.0

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b. Instrumental Settings

Note: Table contains recommended values. Instrumental settings may be adjusted, if necessary, to optimize performance. Subgroups of analytes may be analyzed separately, if necessary, to better tailor conditions for specific analytes.

Analyte	Collision Gas (mTorr)	Collision Energy	Source CID (V)
Desacetyl Cephapirin	1.0	22	7
Amoxicillin	1.0	14	7
DCCD	1.2	22	7
Ampicillin	1.2	18	7
Cefazolin	1.0	18	5
Penicillin G	1.2	28	5
Oxacillin	1.0	17	7
Penicillin V (ISTD)	1.0	15	7
Cloxacillin	1.0	21	6
Nafcillin	1.0	22	6
Dicloxacillin	1.0	20	6

Capillary Temperature 330 °C; use optimized tune files for each analyte.

3. Injection Sequence

- a. Inject external and matrix-matched standards. Verify that all monitored product ions are present in the external standard.
- b. Inject positive and negative controls. Verify absence of analyte carry over in control. If significant carry-over detected, inject solvent/negative control until reduced to acceptable level.
- c. Inject sample extract(s). Include additional washes and standards in run as often as is necessary to ensure proper identification of sample analytes.
- d. Re-inject standard or fortification standard at end of run to verify that instrument response has not deteriorated.

4. Sample Chromatograms

See Section K.2, Sample Chromatograms.

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G. DETECTION AND CONFIRMATION

1. For each injection:
 - a. Plot ion chromatograms for each product ion monitored.
 - b. Determine retention times and abundances for all product ions.
 - c. If multiple product ions are detected for any analyte, calculate the ratios specified below for confirmatory testing.

Analyte	Ion Ratios to Calculate	
Desacetyl Cephapirin	112 / 226	292 / 226
Amoxicillin	114 / 349	208 / 349
DCCD	183 / 241	366 / 241
Ampicillin	160 / 106	192 / 106
Cefazolin	156 / 323	
Penicillin G	160 / 176	
Oxacillin	186 / 287	243 / 287
Penicillin V (Internal Std)	192 / 160	
Cloxacillin	321 / 178	220 / 178
Nafcillin	256 / 199	
Dicloxacillin	254 / 212	256 / 254

2. Screening Requirements
 - a. A sample is screen positive for an analyte if the following criteria are met:
 - i. Average retention time of the product ion peaks in the sample chromatograms matches that found in the external or matrix-matched standard chromatograms within $\pm 4\%$.
 - ii. At least 2 product ion peaks characteristic of the analyte are present with a signal to noise ratio of greater than 3:1. This may be verified by visual inspection.
 - iii. The negative control is negative according to the above criteria.
 - b. Any sample that fails to meet screen positive criteria can be considered screen negative for an analyte if:
 - i. The ISTD added to the sample shows an acceptable screening response.
 - ii. The matrix matched standard shows at least 1 more product ion for that analyte than was detected in the sample.

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3. Confirmation Requirements

- a. Confirmation of an analyte should not be attempted unless the following conditions have been met:
 - i. The ISTD added to the sample shows all monitored ions.
 - ii. The matrix matched standard shows all monitored ions.
- b. Confirmation of analyte identity requires that the following criteria be met:
 - i. Average retention time of the product ion peaks in the sample chromatograms matches that found in the external or matrix-matched standard chromatograms within $\pm 4\%$.
 - ii. All product ions specified for ratio matching are present with a signal to noise ratio of 3 or higher. This may be verified by visual inspection.
 - iii. One of the following ion ratio matching conditions is met:
 - (a) If two product ions are monitored, the presence of one sample ion ratio that matches that calculated for the external or matrix-matched standard within a $\pm 10\%$ arithmetic (not relative) difference.
 - (b) If three product ions are monitored, the presence of two sample ratios that match those calculated for the external or matrix-matched standard within a $\pm 20\%$ arithmetic difference.
 - iv. No confirmable amounts of analyte are detected in the negative control.

4. Criteria for Repeating an Analysis

- a. The conditions described in section G.3. are not met.
- b. The instrument is suspected to be malfunctioning, as demonstrated by: clearly aberrant standard spectra; failure of a calibration check performed shortly after analysis of the sample set; instrumental parameters, especially vacuum readings, outside of normal operating range; or other conditions noted and documented by the analyst.
- c. There is suspected carryover from a previous high concentration sample or standard. In this case, the sample should be reanalyzed after the cause of the carryover has been identified and measures taken to prevent its recurrence.

H. SAFETY INFORMATION AND PRECAUTIONS

- 1. Required Personal Protective Equipment (PPE) - Safety eyewear, protective gloves, and lab coat.
- 2. Hazards

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Procedure Step	Hazard	Recommended Safe Procedures
Antibiotic standards	Some individuals may have allergic reactions to certain β -lactams.	Wear appropriate personal protective equipment to avoid inhalation or dermal contact.
Acetonitrile, Methanol	Flammable	Keep in well-closed containers away from ignition sources. Avoid contact or prolonged exposure to vapors. Work in a fume hood. Keep away from flame or heat.
Formic acid	Corrosive, Caustic	Wear PPE, avoid skin contact.

3. Disposal Procedures

Procedure Step	Hazard	Recommended Safe Procedures
Organic solvents	Flammable	Collect waste in tightly sealed container away from non-compatibles in cool, well ventilated, flammable storage area for disposal in accordance with local, State and Federal regulations.
Formic acid	Strong acid	Neutralize and dispose in accordance with local, State and Federal regulations.

I. QUALITY ASSURANCE PLAN

1. Acceptability Criteria

See Sections G.2-3 for screening and confirmation requirements.

2. Critical Control Points and Specifications

	<i>Record</i>	<i>Acceptable Control</i>
a.	Sample weight	2.0 \pm 0.1 g
b.	Solvent evaporation	38 - 41 °C

3. Readiness To Perform (FSIS Training Plan)

- a. Familiarization

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Note: When performing analyst familiarization, it is expected that for positive samples, Desacetyl Cephapirin, DCCD, Ampicillin, Cefazolin, Penicillin G, and Nafcillin will be confirmed meeting the acceptability criteria described. Amoxicillin, Cloxacillin, Dicloxacillin, and Oxacillin should be detected as screen positive.

- i. Phase I: Standards - Inject external standard solutions in duplicate on at least three different days, and verify instrument response is adequate for confirmatory purposes.
- ii. Phase II: Blanks and Fortified samples - Analyze three sets of six samples consisting of a negative control, positive control, and matrix-matched standard prepared from blank kidney and muscle tissues. Fortify tissues at levels specified in sec. I.7. Sets must be run on different days.

Note: Phase I and Phase II may be performed concurrently.

- iii. Phase III: Check samples for analyst accreditation.
Analyze six check samples fortified at levels between 1 - 2 times the minimum proficiency level (MPL) using analytes and concentrations unknown to the analyst. Any combination of tissues and analytes may be used, and set must include 1 blank.
- iv. Approval from the Supervisor and the Laboratory Quality Assurance Manager (QAM) is required to commence official analysis.

4. Intralaboratory Check Samples

- a. System, minimum contents:
 - i. Frequency: One per week per analyst when samples analyzed.
 - ii. Records are to be maintained for review.
- b. Acceptability criteria:
Refer to I.1 above.
If unacceptable values are obtained, then:
 - i. Stop all official analyses by that analyst.
 - ii. Take corrective action.

5. Sample Acceptability and Stability

- a. Matrices: Bovine muscle and kidney.
- b. Sample receipt, minimum weight: approximately 50 grams.
- c. Condition upon receipt: chilled or frozen.

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- d. Sample storage:
 - i. Time: 30 days.
 - ii. Condition: frozen (< -10 °C).

6. Sample Set

Each sample set must include the following:

- a. Negative control sample (matrix blank).
- b. Positive control sample (fortified matrix blank).
- c. Second matrix blank (for preparation of matrix-matched standard).
- d. Samples to be analyzed.

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7. Sensitivity

a. Minimum proficiency level (MPL):

Analyte	Confirmation MPL (ppb)
Desacetyl Cephapirin	100
DCCD	50
Ampicillin	10
Cefazolin	50
Penicillin G	50
Nafcillin	20

Analyte	Screening MPL (ppb)
Amoxicillin	~10
Oxacillin	~50
Cloxacillin	~10
Dicloxacillin	~50

J. WORKSHEET

The following worksheet is an example.

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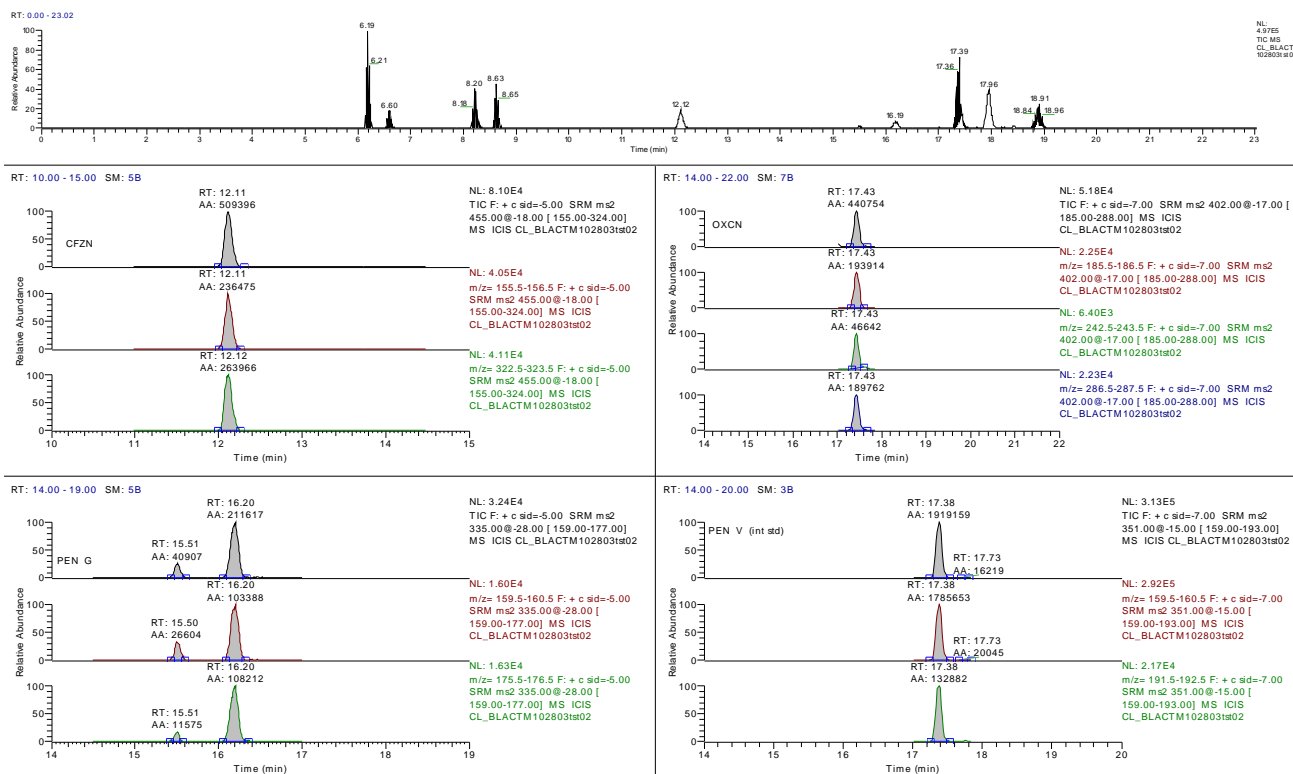
K. APPENDIX

1. References

C.K. Fagerquist, A.R. Lightfield: "Confirmatory analysis of β -lactam antibiotics in kidney tissue by liquid chromatography/electrospray ionization selective reaction monitoring ion trap tandem mass spectrometry," Rapid Communications in Mass Spectrometry **17** (7) 660 - 671 (2003).

2. Sample Chromatograms

Beta Lactam 10 ppb External Standard: Cefazolin, Penicillin G, Penicillin V, Oxacillin.



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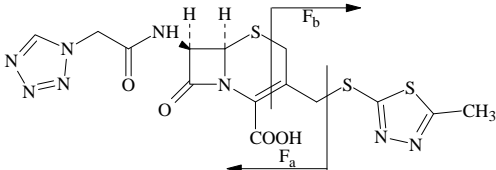
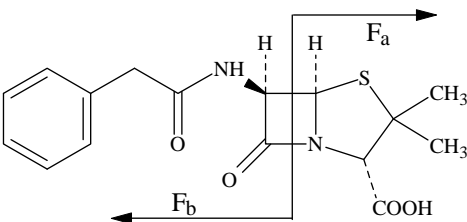
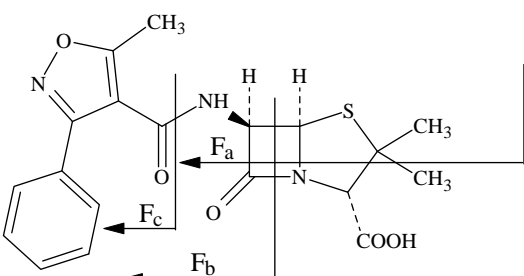
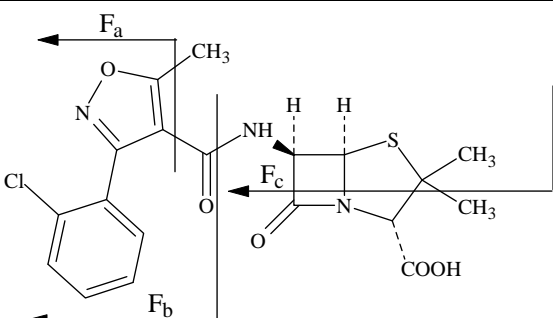
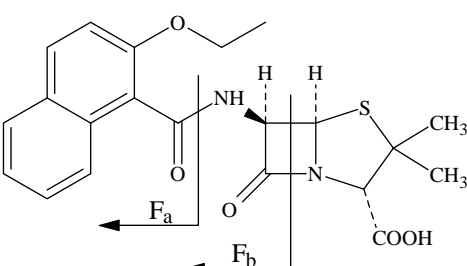
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3. Proposed MS fragmentation

Analyte	Structure	Fragment	Mass
Desacetyl cephapirin	<p>The structure shows Desacetyl cephapirin, a β-lactam antibiotic. Fragmentation pathways are indicated: F_c (loss of the side chain), F_b (loss of the side chain and a portion of the ring), and F_a (loss of the side chain and the entire ring system).</p>	$[M+H]^+$ $[F_a]^+$ $[F_b+H]^+$ $[F_c-CO_2]^+$	382 292 226 112
Amoxicillin	<p>The structure shows Amoxicillin. Fragmentation pathways are indicated: F_c (loss of the side chain), F_b (loss of the side chain and a portion of the ring), and F_a (loss of the side chain and the entire ring system).</p>	$[M+H]^+$ $[F_c+H]^+$ $[F_b+2H]^+$ $[F_a-COOH]^+$	366 349 208 114
Desfuroyl-ceftiofur cysteine disulfide (DCCD)	<p>The structure shows Desfuroyl-ceftiofur cysteine disulfide (DCCD). Fragmentation pathways are indicated: F_c (loss of the side chain), F_b (loss of the side chain and a portion of the ring), and F_a (loss of the side chain and the entire ring system).</p>	$[M+H]^+$ $[F_c+2H]^+$ $[F_b+H]^+$ $[F_a-H]^+$	549 366 241 183
Ampicillin	<p>The structure shows Ampicillin. Fragmentation pathways are indicated: F_c (loss of the side chain), F_b (loss of the side chain and a portion of the ring), and F_a (loss of the side chain and the entire ring system).</p>	$[M+H]^+$ $[F_b+2H]^+$ $[F_a+H]^+$ $[F_c+H]^+$	350 192 160 106

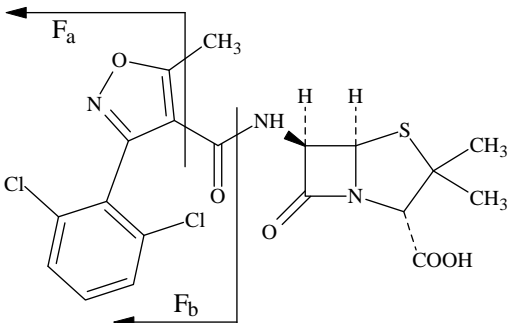
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Cefazolin		$[M+H]^+$ $[F_a]^+$ $[F_b-CH_3]^+$	455 323 156
Penicillin G		$[M+H]^+$ $[F_b+H]^+$ $[F_a+H]^+$	335 176 160
Oxacillin		$[M+H]^+$ $[F_a+H]^+$ $[F_b]^+$ $[F_c]^+$	402 287 243 186
Cloxacillin		$[M+H]^+$ $[F_c+H]^+$ $[F_b]^+$ $[F_a+H]^+$	436 321 220 178
Nafcillin		$[M+H]^+$ $[F_b+H]^+$ $[F_a]^+$	415 256 199

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Dicloxacillin		$[M+H]^+$ $[M+2+H]^+$ $[F_b]^+$ $[F_b+2]^+$ $[F_a+H]^+$	470 472 254 256 212

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L. APPROVALS AND AUTHORITIES

Approvals are on file.

Issuing Authority: Laboratory Quality Assurance Division (LQAD).